to >500/μl and platelets >20,000/μl w/o transfusion were 13 and 14 days, respectively. TRM included 1 pt each with intracranial hemorrhage at d 8, multi-organ failure/sepsis at d 28, IPS/ARDS at d 119, and grade IV GVHD with disseminated adenovirus. 2 of the 4 deaths were in MRD transplants; no serious cases of VOD were observed. Grade 2–4 GVHD was seen in 5/16 evaluable MUD pts and 3 of 29 (all sex mismatched) evaluable MRD pts. CMV reactivation was seen in nearly all CMV sero+ pts; bk activation was seen in 25 pts and hemorrhagic cystitis in 10. One case of PTLD, 3 graft failures, and adenovirus infection with nephritis, cystitis, and transient cardiomyopathy in one patient, and fatal hepatitis in a second patient were also seen. Relapse/# evaluable for different diseases are: AML 12/26 (46%), imatinib-resistant CML 3/3 MDS 0/5, MM 3/3, NHL 0/3, ALL 0/3, CLL 0/2. Relapse rates in evaluable MRD pts, 15/29 (52%), were statistically higher compared to MUD pts, 3/16 (19%) (p = .055); marrow (2/8) vs BSC, (16/37), were comparable. Alemtuzamab has been effective at controlling GVHD but infectious complications are common and high relapse rates preclude routine use of this approach in MRD patients with advanced myeloid diseases. Current use of this agent is depending on donor (MRD vs MUD) and disease (myeloid vs other) status.

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MISSING KILLER IMMUNOGLOBULIN-LIKE RECEPTOR (KIR) LIGAND CONFERS PROTECTION FROM RELAPSE IN RECIPIENTS OF UNRELATED HEMATOPOIETIC CELL TRANSPLANTATION (HCT) FOR AML

Hsu, K.C.[1]; Malkki, M.[2]; Gooley, T.A.[2]; Dupont, B.[3]; Petersdorf, E.W.[2], International Histocompatibility Working Group HCT-KIR Component. 1. Memorial Sloan-Kettering Cancer Center, New York, NY; 2. Fred Hutchinson Cancer Research Center, Seattle, WA; 3. Sloan-Kettering Institute for Cancer Research, New York, NY.

The relationship between donor inhibitory KIR and recipient HLA has been proposed as the basis for KIR-driven alloreactivity by donor natural killer (NK) cells, leading to higher overall survival (OS) and a reduction in relapse, graft-versus-host disease (GVHD) and graft rejection in high-risk acute myelogenous leukemia (AML) patients undergoing HLA-mismatched transplants. Previous analyses of KIR effects on HLA-mismatched HCT have applied a "KIR ligand incompatibility model," which predicts NK alloreactivity when donors with class I ligands for inhibitory KIR are paired with recipients lacking the particular class I ligand group. We have developed an algorithm that recognizes that population frequencies for inhibitory KIR2DL2/3, -2DL1, and -3DL1 are close to 100%, whereas the corresponding class I ligands (HLA C1-group, C2-group, or Bw4) have population frequencies that deviate greatly from 100%, leading to the frequent situation of "missing KIR ligand," even in HLA-matched transplants. The "missing KIR ligand model" was applied in an analysis of 1765 unrelated donor HLA-A, -B, -C, -DRB1, and -DQB1 matched and mismatched HCT pairs from the IHWG. All patients received myeloablative conditioning followed by infusion of a T-replete allograft. Using the "missing KIR ligand model," KIR ligand absence/presence was correlated with overall survival (OS) and relapse (Table). There was no benefit in OS or relapse for ALL or CML patients lacking KIR ligand. In contrast, AML patients lacking KIR ligand demonstrated a significantly lower relapse rate (p=0.001), that could be exclusively attributed to the lack of the HLA C2-group ligand for donor KIR2DL1 (p<0.0001). The lower relapse likely contributes to the higher OS (p=0.04) seen in these patients. Comparison of the "missing KIR ligand" and "KIR ligand incompatibility" algorithms was then performed for AML/MDS (n=176), ALL (n=137), and CML (n=320) patients and unrelated donors mismatched at HLA-B and/or HLA-C. Neither model showed a benefit in OS or relapse for any disease group with exception of the missing ligand model in AML for relapse (p=0.008) This study indicates that lack of HLA C2-group ligand in the patient with AML is associated with a lower risk of relapse and improved overall survival, implying that release from KIR2DL1 inhibition enhances donor NK alloreactivity against AML. Adoptive immunotherapy with donor NK infusion may be particularly beneficial in these patients.

Missing KIR Ligand Algorithm and Disease-Specific Outcome

	Overall Mortality		Relapse	
	Hazard Ratio	p-Value	Hazard Ratio	p-Value
AML/MDS				
All ligands present (n = 105)	1	—	1	—
Any KIR ligand absent (n = 373)	0.86	0.27	0.55	0.001
HLA C2-Group ligand absent (n = 149)	0.73	0.04	0.34	<0.0001
CML				
All ligands present (n = 230)	1	—	1	—
Any KIR ligand absent (n = 683)	1.1	0.39	1.08	0.68
ALL				
All ligands present (n = 77)	1	—	1	—
Any KIR ligand absent (n = 297)	1.12	0.49	1.17	0.56

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TRANSFUSIONAL IRON BURDEN AFTER BONE MARROW TRANSPLANTATION

Jastaniah, W.A.[1]; Harmatz, P.[2]; Pakbaz, Z.[2]; Singer, T.[2]; Fischer, R.[2]; Vichinsky, E.[2]; Walters, M.C.[2] 1. British Columbia's Children's Hospital, Vancouver, BC, Canada; 2. Children's Hospital Oakland, Oakland, CA.

Background: The significance of transfusional iron overload post-BMT for sickle cell disease (SCD) and hematological malignancies has not been well defined. Hence, we performed tests to assess iron burden before and after BMT that included: serum iron, ferritin, TIBC, and liver iron concentration (LIC) by liver biopsy and/or SQUID biosusceptometer (Ferritometer®, Model 5700, Tristan Technologies, San Diego) under the standardized Hamburg-Torino-Oakland protocol. We compared results in children with SCD and AML to those with thalassemia major (TM). **Results:** Fifteen children [SCD (N=4), TM (N=6), and AML (N=5)] received conventional myeloablative allografting at Children's Hospital Oakland (2000–2003). The mean (±SD) age (y) at BMT was 10 ± 2.3, 6.8 ± 5.1, and 5.4 ± 2.9 among SCD, TM, and AML patients, respectively. The mean duration of transfusion exposures was 2.5, 5, and 2 years, respectively. Results of pre-BMT iron burden are summarized in the Table. Pre-BMT liver histology was assessed in 6 patients (SCD 1 and TM 5). Inflammation was present in 4 TM patients and portal fibrosis in 1 SCD and 3 TM patients. Hepatic venoocclusive disease (VOD) developed in 5/15 patients. TM patients were more likely to develop VOD (80 vs 20%) and pre-BMT, nonspecific inflammatory liver changes were observed in 60% of TM patients (versus 10% of others). Post-BMT, six patients were treated by regular phlebotomy. There was poor correlation between serum ferritin and LIC. The serum ferritin and WBC normalized by 40 months among phlebotomized patients. However, normalization of LIC required a longer period of time. The amount of iron removed by phlebotomy correlated with the change in LIC as measured by SQUID. **Conclusion:** Pre-BMT, we observed a significant iron burden in children with SCD, TM, and AML, which, if untreated, persisted post-BMT. Liver inflammation and VOD was increased in TM patients, which might reflect liver injury that is influenced by the duration of exposure rather than simply by the magnitude of iron overload. Phlebotomy is effective in unloading iron post-BMT in SCD and TM patients. This pilot study showed that serum ferritin is an unreliable indicator of iron overload post-BMT. The significance of transfusional iron overload in patients with hematological malignancies remains uncertain, but this study suggests that there is potential for hepatic injury post-BMT. Ongoing studies are being conducted to better define the natural history of iron overload in post-BMT patients.

Iron Burden Before BMT (Mean ± SEM)

Disease	N	Serum Iron (μg/dl)	Ferritin (ng/ml)	TIBC (μg/dl)	ALT (U/L)	RBC (ml/kg)	LIC (mg Fe/gm liver dry wt)
TM	6	165 ± 301	1964.3 ± 180	214 ± 16.8	141.7 ± 67.1	561.7 ± 277.4	15.1 ± 1.4
SCD	4	146.5 ± 46.3	2124.5 ± 1053	233 ± 36.3	67.3 ± 39.4	200.8 ± 106.9	14.7 ± 7.3
AML	5	96 ± 0	2229.5 ± 785.5	241 ± 59	31 ± 1.6	311.1 ± 114.8	ND

TM, Thalassemia major; SCD, Sickle cell disease; AML, acute myelogenous leukemia; TIBC, total iron binding capacity; ALT, alanine transaminase; RBC, volume RBC transfused; LIC, Liver iron concentration.

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DESCRIPTIVE ANALYSIS OF CHANGING TOXICITY PROFILES FOR 30 AND 100 DAY MORTALITY AFTER MATCHED RELATED DONOR (MRD) ALLOGENEIC (ALLO) HEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT) USING PO VS IV BUSULFAN (BU) BASED CONDITIONING REGIMENS

Gupta, S.; Cole, S.W.; Salzman, D.E.; Ashraf, K.; Aggarwal, C.; Vaughan, W.P. Bone Marrow Transplantation Program, University of Alabama at Birmingham, Birmingham, AL.

We have been using BU based preparative regimens in ALLO MRD HSCT for hematological malignancies since 3/92. Prior to 3/99, 135 pts were treated with po BU in BU/Cy2, BU/Cy/VP16, and BU/Fludara preparative regimens (po group). Between 3/99 and 5/04, 97 pts received iv BU with pharmacokinetic (PK) guided dosing in one of the same three regimens (iv group). The distribution of diagnoses was similar in the two groups. In the po group the diagnoses were acute Leukemia (47), NHL (38), CML (37), HD (10) and other (3). In the iv group the diagnoses were acute Leukemia (34), NHL (32), CML (20) HD (3) and other (8). The female/male ratio was also similar in the two groups (po 63/72, iv 44/53). The pts in the iv group were older (>45 yrs) (63/97 vs 57/135, p=0.0006, χ^2), had a slightly higher ASBMT relapse risk score (72% vs 66%, I/HR), a higher ratio of ASBMT HR CML pt (11/20 vs 12/37) and more pts with less than 10/10 match (8% vs 3%). Despite the higher risk for both relapse and transplant related mortality in the iv group, the 30 and 100 d mortality was similar for the two groups (4% and 31% for iv BU: 8% and 28% for po BU). Defining cause of death as the initiating event or first organ failure that led to death, we found that, not unexpectedly, death due to Hepatic Veno-Occlusive Disease (HVOD) was eliminated with the use of PK directed iv BU (0/21 vs 8/34, p=0.014, χ^2). GVHD/immunodeficiency and cardio-pulmonary toxicity were not increased in the higher risk iv group, but these two causes remained significant and together accounted for 67% of the overall non relapse mortality in the combined population. However, death due to progression of primary disease was greater in the iv group (9/30 vs 4/38, p=0.04, χ^2) reflecting their greater ASBMT relapse risk. Because of elimination of HVOD and no increase in any other cause of non-relapse mortality as a result of pharmacokinetic guided iv BU targeting, we are now able to transplant higher risk pts without an increase in the 30 or 100 d mortality. These results are a part of incremental multiple center pharmaceutical and supportive care efforts directed towards increasing the value of myeloablative transplants to older and higher risk pt (Table 1).

Cause of Death—100 d Mortality by Route of BU Administration

	Progressive Disease	Veno-Occlusive Disease	GVHD/ Immunodeficiency	Cardio-Pulmonary	Others
po	4	8	15	7	4
iv	9	0	7	8	6

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THE REQUIREMENT OF MHC CLASS II FOR DONOR CD4$^+$CD25$^+$ T CELL-MEDIATED SUPPORT OF EARLY ALLOGENEIC PROGENITOR CELL ENGRAFTMENT

Hanash, A.M.; Levy, R.B. University of Miami School of Medicine, Miami, FL.

CD4$^+$CD25$^+$ regulatory T cells have recently emerged as a potential modality for suppression of GVHD and promotion of allogeneic engraftment. In a fully allogeneic MHC mismatched model utilizing C57BL/6 (B6) T cell-depleted bone marrow (BM-TCD) and sublethally conditioned (7.0 Gy TBI) BALB/c recipients, we have demonstrated that donor CD4$^+$CD25$^+$ T cells are capable of supporting multi-potential and lineage-committed donor progenitor activity as well as long-term chimerism and tolerance. In order to pursue the potential for clinical application, we sought to determine molecular and antigenic requirements involved in the initial promotion of allogeneic engraftment. Although an essential role for IL-10 has been demonstrated in certain regulatory T cell models, augmentation of donor progenitors seven days post-BMT did not require CD4$^+$CD25$^+$ T cell IL-10: co-transplantation of 1×10^6 B6-wt or B6-IL-10$^{-/-}$ CD4$^+$CD25$^+$ T cells both significantly increased GFP$^+$ splenic CFU-GM when transplanted with 2×10^6 B6-GFP BM-TCD into BALB/c recipients (mean CFU ± SE: BM alone, 657.5 ± 248.2; BM + wt, 1972 ± 331.5; BM + IL-10$^{-/-}$, 1965 ± 401.7; both p<.05 vs. BM alone). Assessment of the antigenic requirements for activation of progenitor support demonstrated that donor CD4$^+$CD25$^+$ T cells did not require alloreactivity to support progenitors, as BALB/c × B6 F1 CD4$^+$CD25$^+$ T cells significantly increased B6 CFU-GM in BALB/c recipients (p<.001 vs. BM alone). However, B6 CD4$^+$CD25$^+$ T cells failed to augment MHC-disparate C3H/HeJ CFU-GM in BALB/c recipients (p>.05 vs. BM alone), suggesting that donor CD4$^+$CD25$^+$ T cells might require recognition of syngeneic MHC for progenitor support. Indeed, augmentation of donor CFU-GM was abrogated when B6 CD4$^+$CD25$^+$ T cells were co-transplanted with B6-MHC class II$^{-/-}$ marrow into BALB/c recipients (p>.05 vs. BM alone). In conclusion, donor CD4$^+$CD25$^+$ T cells capable of promoting long-term engraftment and tolerance do not require IL-10 for support of initial donor progenitor activity, however progenitor support does require co-transplantation with syngeneic MHC class II-expressing marrow. Strikingly, there may be distinct antigenic requirements for promotion of engraftment and suppression of GVHD, suggesting that the CD4$^+$CD25$^+$ T cells responsible for these effects may be distinct as well. Further determination of the role of donor MHC class II may prove essential for proper construction of CD4$^+$CD25$^+$ T cell-based allo-engraftment protocols.

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ABSENCE OF CD80/86 EXPRESSION ON DONOR BONE MARROW IS SUFFICIENT TO CIRCUMVENT T CELL MEDIATED RESISTANCE FOLLOWING EXPERIMENTAL "MINI" MHC-MATCHED ALLOGENEIC BMT

Zimmerman, Z.F.; Levy, R.B. University of Miami School of Medicine, Miami, FL.

Successful MHC-matched allogeneic BMT following non-myeloablative conditioning requires overcoming immune mediated resistance by alloreactive host T cells to achieve engraftment. Manipulation of donor APC's may be a clinically feasible strategy to achieve this aim. In the current study, donor BM from CD80/86$^{-/-}$ mice was transplanted to determine the requirements of donor APC in eliciting resistance in pre-clinical "mini" (5.5 Gy TBI) BMT models. Non donor antigen sensitized ("naïve") and donor antigen sensitized ("memory") recipients were transplanted. "Naïve" C3H.SW (H-2b) mice were transplanted with 4×10^6 TCD-BMC from B6-wt or CD80/86$^{-/-}$ donors. All mice receiving B6-wt BM displayed brief donor cell presence peaking at day 7–10 post-BMT followed by disappearance (i.e. rejection) 4–5 weeks later. In contrast, C3H.SW mice receiving CD80/86$^{-/-}$ marrow achieved and maintained significant donor chimerism (>50%) in both B cell and importantly, T cell compartments.